Multiple functions of photosystem II

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The most important function of photosystem II (PSII) is its action as a water-plastoquinone oxido-reductase. At the expense of light energy, water is split, and oxygen and plastoquinol are formed. In addition to this most important activity, PSII has additional functions, especially in the regulation of (light) energy distribution. The downregulation of PSII during photoinhibition is a protection measure. PSII is an anthropogenic target for many herbicides. There is a unique action of bicarbonate on PSII. Decrease in the activity of PSII is the first effect in a plant under stress; this decreased activity can be most easily measured with fluorescence. PSII is a sensor for stress, and induces regulatory processes with different time scales: photochemical quenching, formation of a proton gradient, state transitions, downregulation by photoinhibition and gene expression.

The light reactions of photosynthesis are the basis of life on earth. While the bacterial light reaction and photosystem I (PSI) were developed first, the oxygenic photosystem II (PSII) of plants, algae, cyanobacteria and prochlorophytes made the process of photosynthesis more durable, using water as electron donor. Photosystem II can be considered as a water-plastoquinone oxido-reductase. Using light energy, water is split, and oxygen and plastoquinol are formed. The transformation of light energy into chemical energy is the primary function of PSII. During evolution PSII has developed several other functions which are discussed in this paper.

The basic function of the D1 and D2 proteins in positioning the cofactors of photosystem II

The PSII reaction center1,2 contains six polypeptides: D1, D2, a heterodimer of cytochrome b559 and the gene products of psbI and psbW. The central core of the PSII reaction center consists of the D1 and D2 proteins where all the active components are embedded (Fig. 1). The function of the D1 and D2 proteins is to keep all cofactors in such a position that the reactions can take place in the right order. After light absorption by the light harvesting complexes, the exciton energy is transferred via the accessory chlorophylls to the \( P_{880} \) in the chlorophyll special pair. Charge separation takes place: an electron is transferred from \( P_{880} \) to pheophytin. The electron hole of the oxidized \( P_{880} \) is filled by the tyrosine Y161 which is located in the D1 protein; this tyrosine extracts an electron from water via the water oxidizing complex, which includes a tetra-manganese cluster. The reduced pheophytin carries an electron to the primary quinone electron acceptor of PSII, QA; the electron is then forwarded to QA, the secondary quinone electron acceptor of PSII. After two turnovers the Qs becomes reduced to \( Q_{sH2} \) which then escapes from the reaction center complex into the thylakoid membrane and is named plastoquinol. Oxidized plastoquinol takes the place of QB.

The unique role of bicarbonate

Carbon dioxide is required for photosynthesis. It is fixed by ribulose 1,5-bisphosphate carboxylase and further reduced to carbohydrate. However, CO2 is also involved in the photosynthetic electron transport of plants, algae and cyanobacteria; it is called the bicarbonate effect. This effect is specific for PSII and does not occur in the reaction centers of anoxygenic photosynthetic bacteria. The phenomenon includes decreased activity of PSII (any reaction between water oxidation and the reduction of plastoquinone) upon the depletion of CO2/bicarbonate, which is restored by the addition of bicarbonate. Warburg and Krippah3 were the first to show that CO2 accelerates the production of oxygen on illumination of isolated chloroplasts in the presence of an electron acceptor like ferricyanide. This phenomenon was experimentally dif-
difficult to study until Stemler and Govindjee\textsuperscript{4} developed a dependable method to investigate the bicarbonate effect. Since then, many research groups have studied this effect. It can be seen at the level of the intact leaf as well as in isolated reaction center preparations. The requirement is located in PSII, both at the acceptor and at the donor side.

The first indication for a bicarbonate effect on the acceptor side of PSII was obtained by Wydrzynski and Govindjee\textsuperscript{5} who measured Chl \( \text{a} \) fluorescence induction kinetics in thylakoids after CO\(_2\)-depletion and after re-addition of bicarbonate. Depletion led to a faster fluorescence rise, as is also the case when a herbicide like diuron (DCMU) is added to block electron flow at the acceptor side of PSII. Studies using mutants revealed that there are two binding sites for bicarbonate at the acceptor side of PSII (Fig. 1): one is located at the non-heme iron between \( Q_{\text{A}} \) and \( Q_{\text{B}} \), the second one is in the positively charged region of the \( Q_{\text{B}} \) binding site, most probably arginine 257 of the D1 protein. Bicarbonate is suggested to function in the protonation of reduced \( Q_{\text{B}} \) and in the stabilization of the \( Q_{\text{A}} \)-Fe-\( Q_{\text{B}} \) structure.

A role for bicarbonate at the donor side of PSII was proposed from the beginning by Stemler\textsuperscript{6}. Recently, the group of Klimov\textsuperscript{7} has published much evidence that this effect on the donor side is located in the water-oxidizing complex of PSII. After depletion of PSII particles from manganese, bicarbonate was found to be essential for the early steps of photoactivation of the oxygen evolution complex when added together with manganese. Klimov \textit{et al.}\textsuperscript{7} suggested that bicarbonate may serve as a ligand to Mn (Fig. 1). (Reference 1 may be seen for older references and a recent review\textsuperscript{8}, on the bicarbonate effect).

The anthropogenic function as a target for herbicides

The niche enclosed by the 4\textsuperscript{th} and the 5\textsuperscript{th} transmembrane helices and the parallel helix of the D1 protein of PSII is the binding site of the cofactors including \( Q_{\text{B}} \). Man has taken advantage of the phenomenon that this niche can bind many chemicals, including those inhibiting electron flow. There are many chemically different plant-killing compounds acting at PSII, as the urea-type herbicides like diuron (DCMU) and the
triazines like atrazine. In order to understand the mechanism of action of these herbicides, a basic knowledge of the "two-electron gate" action of QA is required. While QA is permanently bound to the D2 protein and acts as a single electron carrier, QB is reversibly bound to the D1 protein. While bound to the D1 protein, QB first accumulates two electrons and two protons to form QBH2 and then escapes from its binding site. In its oxidized and reduced state, QB and Q8H2, it is only weakly bound and can easily leave its binding site; the semiquinone QB- is more tightly bound. It is widely accepted that the mechanism of reversible binding to the protein instead of QBH, instead of QA and electron transport beyond this point is thereby prevented. It may be remarked that plants are only slowly killed by these type of herbicides; plants die from starvation because of absence of photosynthetic activity and from secondary reactions like bleaching of the chlorophyll. Many references on this topic can be found in the reviews by Van Rensen and by Oettermeier.

Regulation of light energy dissipation

When a plant is exposed to an extreme high light intensity, it cannot run away. Therefore it has developed regulatory mechanisms to dissipate the excess light energy. Although a plant is not able to move to a shaded area, many plants can change the position of their leaves in order to avoid too much irradiation. If a change of leaf position cannot prevent the plant from receiving an excessive amount of light energy that cannot all be converted by photochemistry (photosynthesis), the plant has to take other measures to avoid possible damage to the photosynthetic apparatus.

There are several mechanisms by which plants can convert or dissipate excessive irradiation through non-radiative energy dissipation. Examples of these mechanisms are heat emission and photorespiration. Photorespiration occurs in C3 plants and can be described as an overflow of reductive energy which is initiated by the reaction of Rubisco with oxygen. Two more processes are the xanthophyll cycle and photoinhibition. Another important photoprotective process is fluorescence quenching.

Most of these mechanisms aim to dissipate light energy before it can harm the plant. Such mechanisms are active at a much shorter timescale compared to long-term adaptation to high irradiance which involves a change in the balance between the synthesis and degradation of proteins and pigments. Additional environmental stress, such as temperature and water stress, lower the photosynthetic rate and can enlarge the degree to which absorbed light becomes excessive, increasing the need for energy dissipation. Earlier research has shown that plants, next to changing the photosynthetic capacity, alter the activity of energy dissipative pathways and pigment-protein composition, besides changing the morphological structure of their leaves.

The first response of a plant upon stress is a decreased activity of PSII. Curwiel and Van Rensen used a triazine-resistant biotype of Chenopodium album as a model plant to study the interrelated effects of a decreased level of PSII activity on fluorescence, xanthophyll cycle, state transitions and photoinhibition. These triazine-resistant (R) plants have a mutation in the D1 protein: at site 264, serine is altered to glycine. Because of this mutation, the R plants are not only unable to bind the herbicide atrazine, but also have a 3-fold lower rate of electron flow between QA and QB (in the absence of herbicide). Thus, the R plants have an intrinsic lower activity of PSII. In addition, the chloroplasts of these resistant plants have shade-type characteristics: more and larger grana, more LHC (Light Harvesting Chlorophyll) associated with PSII and a lower chlorophyll a/b ratio. The thylakoid membranes of the R type chloroplasts contain less coupling factor and they utilize the pH gradient less efficiently for photophosphorylation than do the S ones.

Curwiel and Van Rensen observed that resistant plants have a lower Fv/Fm value (variable over maximal fluorescence, indicating the potential quantum yield of PSII). This lower Fv/Fm value can be observed in Fig. 2-4, where the effect of a photoinhibitory treatment is expressed as a function of the Fv/Fm value. The most right-hand side points in the figures are the values before such a treatment (controls); the R plants have always a lower Fv/Fm ratio. In Fig. 2-4 results are presented of experiments in which leaves of triazine-resistant (R) and wild-type (S, sensitive) Chenopodium album plants are given a...
Fig. 2—Effect of a photoinhibitory treatment on the photochemical quenching (qP) of leaves of triazine-resistant (R) and sensitive (S) Chenopodium album plants. [qP is shown as a function of the level of photoinhibitory damage, expressed as Fv/Fm. The bars in the figures indicate ± SD (n = 2-32); SD bars not showing are within the point markers].

Fig. 3—Effect of a photoinhibitory treatment on the fraction of closed reaction centers (1-qP; this is related with the concentration of reduced QA) of R and S leaves of C. album. [(1-qP) is shown as a function of the level of photoinhibitory damage, expressed as Fv/Fm. For further details, see legend of Fig. 2].

Fig. 4—Effect of a photoinhibitory treatment on the non-photochemical quenching (qN) of R and S leaves of C. album. [qN is shown as a function of the level of photoinhibitory damage, expressed as Fv/Fm. For further details, see legend of Fig. 2].

One of the photoprotection mechanisms in chloroplasts involves non-radiative energy dissipation which is measurable as non-photochemical fluorescence quenching, qN. This quenching results from the non-photochemical de-excitation of the singlet excited state of chlorophyll associated with PSII. qN includes three major components which have different relaxation kinetics. The first component is qE which is high energy state quenching, related to the light-driven proton gradient across the thylakoid membrane. It has relaxation kinetics in one to two minutes range. A second component is qT, quenching related to state 1
- state 2 transitions\(^{25}\), regulated by phosphorylation of LHCII and with relaxation kinetics in the timescale of 5-10 minutes. The third component is the quenching, qI, due to photoinhibition\(^{26,27}\). It has relaxation kinetics in the order of hours. The major component of qN is qE which is caused by thermal dissipation of excess light energy in the pigment bed. qE is considered to be an important mechanism for photoprotection of plants \(\text{in vivo}^{28}\).

Curwiel\(^{20}\) observed that qN, before and after a period of photoinhibitory treatment, is lower in the R biotype (Fig. 4). Thus, R plants have a lower capacity of non-radiative energy dissipation. The differences between the two biotypes become smaller at a higher level of photoinhibitory damage. After analyzing the contributions of the three components of qN, it appeared that the lower qN is mainly due to a lower qE in the R biotype. There was a little higher qT, and qI was a little bit lower in the R plants. It was also reported\(^{19}\) that the resistant plants have more light-induced zeaxanthin formation and a larger change in light scattering than the wild-type plants. These results lead Curwiel\(^{20}\) to propose a model for the regulation of photosynthesis and energy dissipative pathways in resistant and susceptible plants of Chenopodium album (Fig. 5). In this scheme, the activity of PSII is at the basis of the regulatory pattern. The conclusion of this work is that the lower activity of PSII in resistant plants leads to adaptation of the plants in the direction of shade-type chloroplasts (more LHC connected with PSII). The combination of shade-type characteristics with a lower electron flow rate between QA and QB leads to lower qP and qE in the R plants. As a consequence, the R plants are less able to cope with situations of excess light energy, leading to more photoinhibitory damage of the photosynthetic apparatus compared to the sensitive plants, as was actually reported\(^{18,29}\).

**Conclusion: a model for the adaptation of a plant to light intensity**

A plant is dependent on light for its survival. The mature leaf is permanently adapting to the intensity of the light to which it is exposed. There are several adaptations of the plant: optimizing activity of processes as well as structural changes. The several adaptations have different timescales, from microseconds to hours:

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<\mu s (qP) \rightarrow s (qE) \rightarrow \min (qT) \rightarrow 1-2 \text{ hours (qI)} \rightarrow \text{hours (gene expression)}
\]

We suggest that these regulatory activities are all initiated by processes in PSII. Melis\(^{30}\) proposed that high light intensity, large antenna size, limitation of CO\(_2\), and suboptimal temperature cause a higher reduction level of QA, followed by more non-assimilatory diss-
pation, more charge recombination and a higher probability for D1 photodamage. In our view, the conditions mentioned by Melis, may be extended to all conditions of biotic and abiotic stress. Under stress, a plant has a lower activity of photosynthesis, leading to a higher level of reduced QA. When QA is more reduced, less photosynthetic charge separation can take place, leading to a lower photochemical quenching (qP), in the timescale of up to a μs. In the seconds timescale the high level of reduced QA leads to more reduction of the plastoquinone pool, a high proton gradient over the thylakoid membrane and a high energy dependent quenching, qE. The high reduction level at and around plastoquinone activates the LHCII kinase, which phosphorylates the LHCII, causing a change in protein recognition that results in redistribution of energy to PSI (photosystem I) at the expense of PSII (see Allen31 as an example of many reviews on this topic). This transition from light-state 1 to light-state 2 leads to lower fluorescence of PSII and a lower qT in the minutes timescale. At longer timescale damage to the D1 protein is building up, leading to photoinhibition, qL. Allen31,32 proposed that the redox level of the plastoquinone pool and the cytochrome b6/f complex between PSI and PSII controls de novo protein synthesis, assembly and breakdown. Evidence for such a molecular redox signalling, the coupling of gene expression to electron transfer, has now been reported by several groups33-36. This dynamic adjustment of gene expression leads to adaptation to the prevalent light intensity by altering the composition and structure of the photosystems and the photosynthetic apparatus. Thus, activity of PSII may act as a sensor for stress and, via regulation of the redox level of the PQ pool, initiates different adaptations by the control of gene expression at different levels.

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